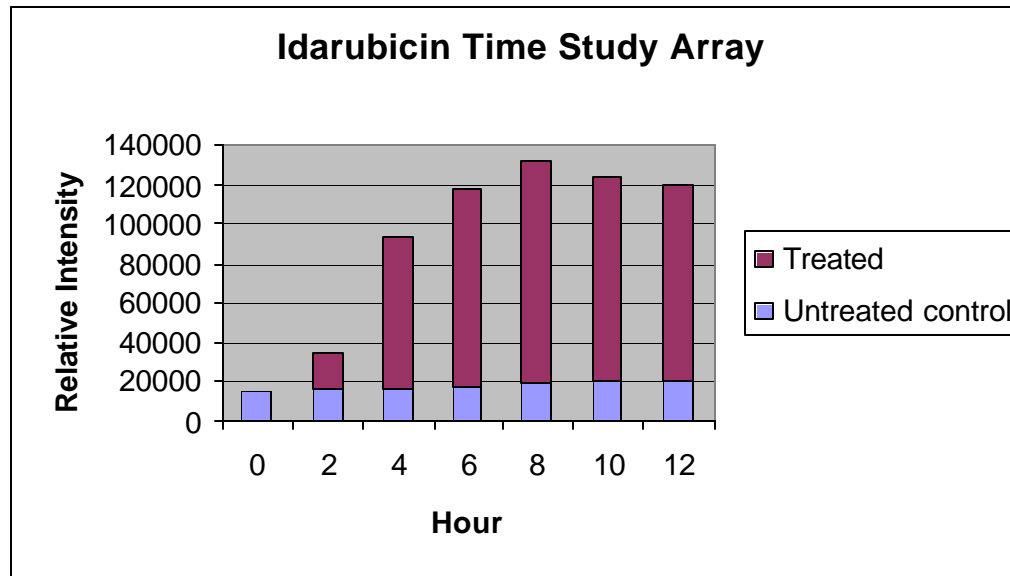


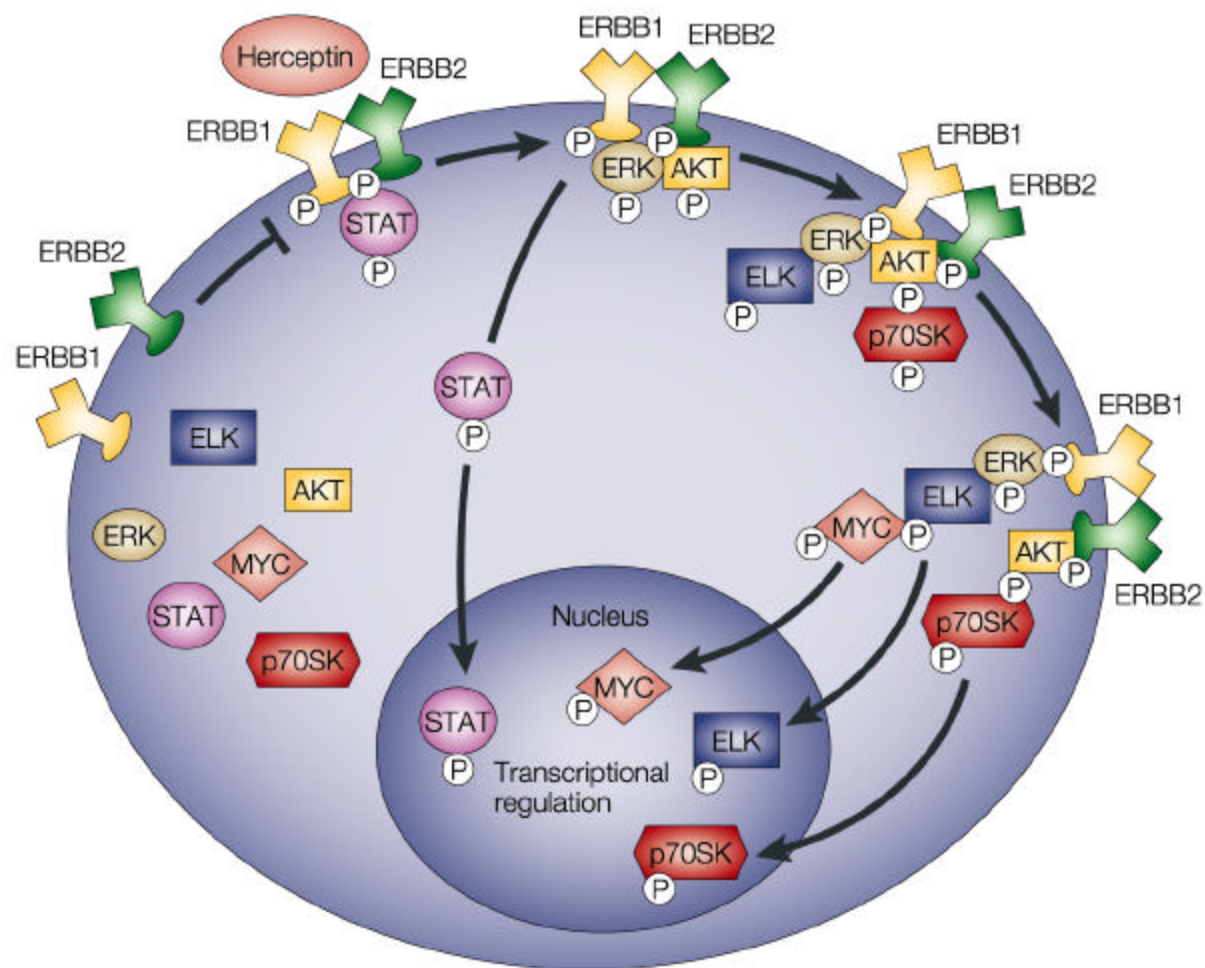
Human B Lymphoma Apoptosis Pathway Protein Microarrays

Cleaved Caspase 3

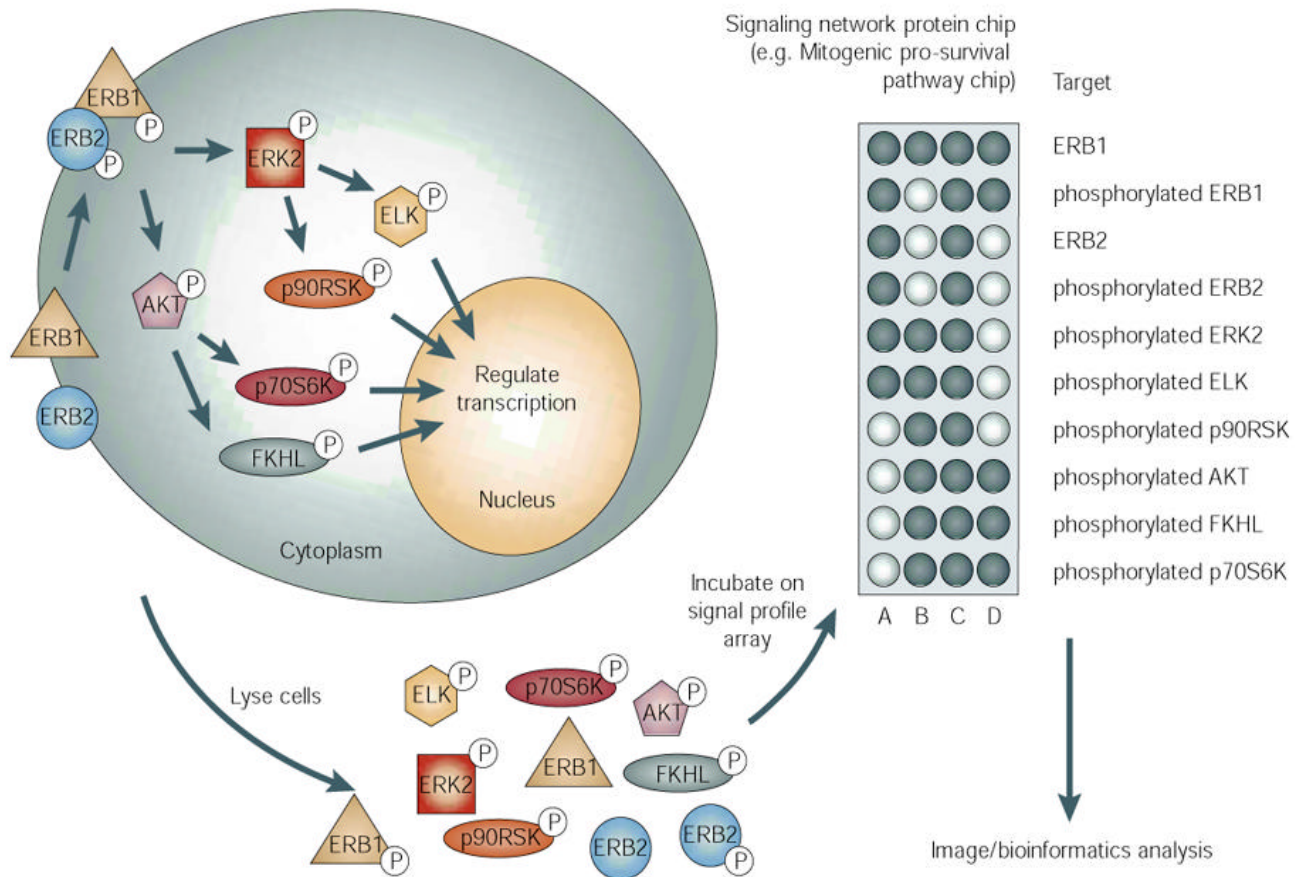


Untreated Treated



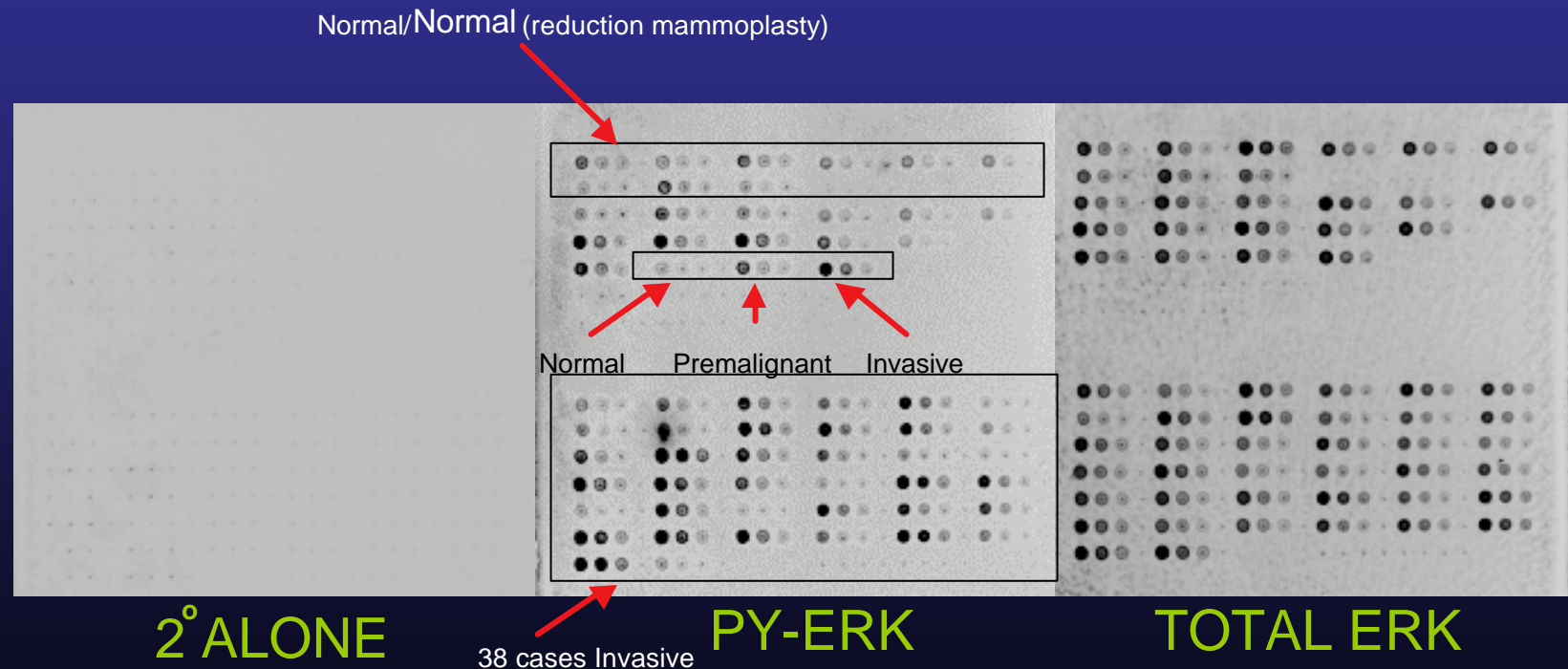


Signal Transduction Pathway Profiling



Use of Novel Protein Array Technology: Signal Pathway Profiling in Human Breast Cancer Biopsy Specimens

Coupling Laser Capture Microdissection With True Signal Pathway Profiling

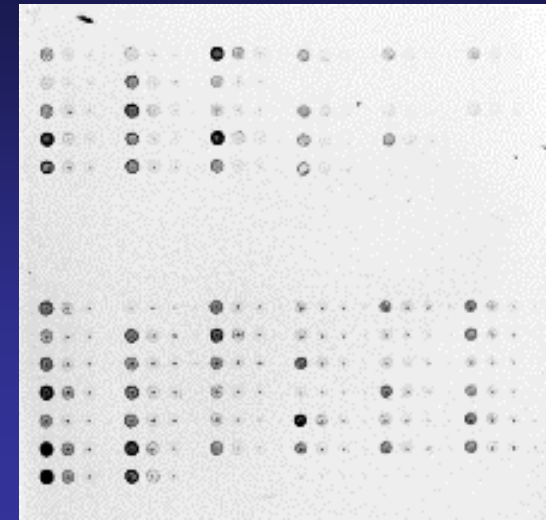
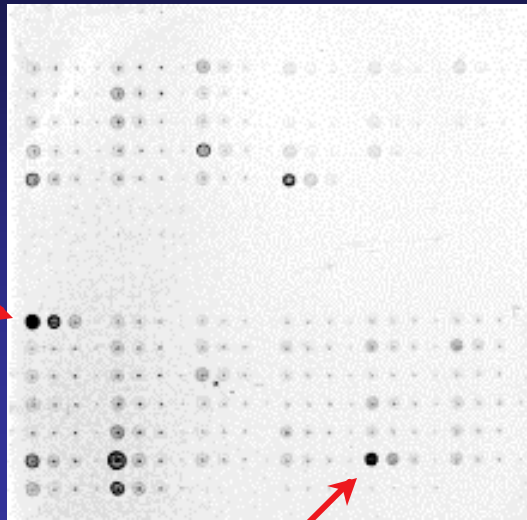


Ongoing work: cluster analysis with 135 phospho-specific endpoints, all normalized to the self protein for true signal pathway profiling

Phospho-AKT (ser 473)

TOTAL AKT

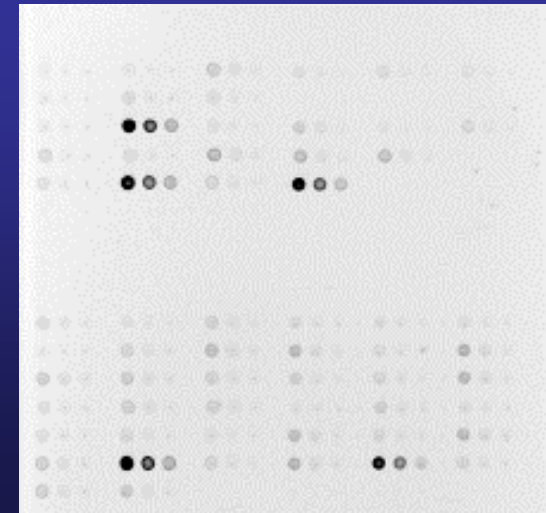
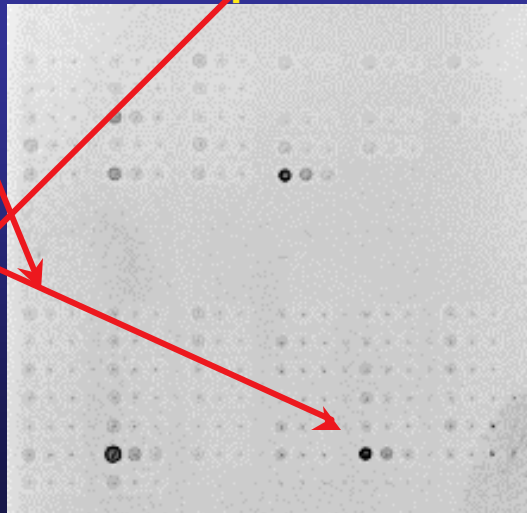
Discordant activation



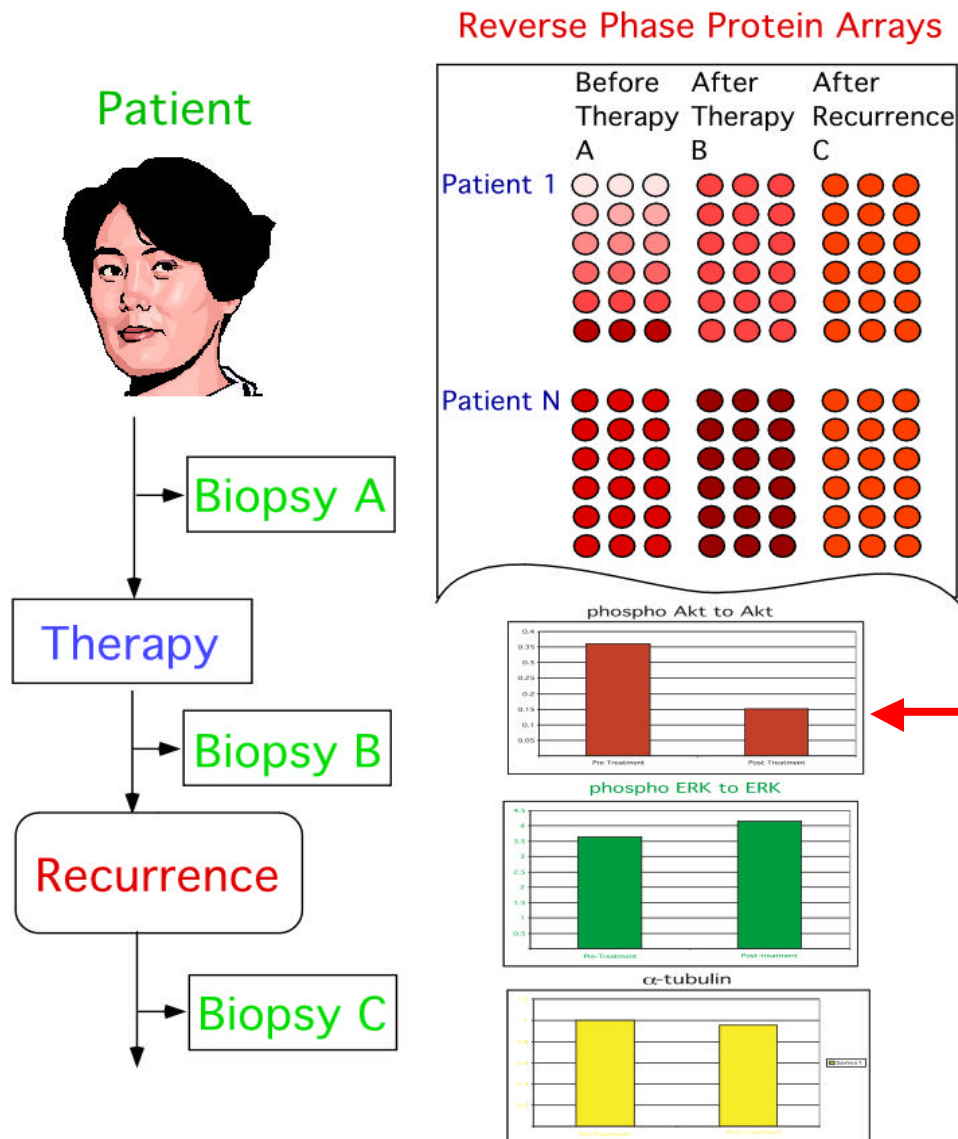
Phospho-ERB2

Total ERB2

Concordant activation



Clinical Trial Molecular Target Analysis



Clinical Trial:

- Herceptin followed by Taxol
- Metastatic Breast and Ovarian Ca
- Findings to date

HERCEPTIN REDUCES P-Akt PROSURVIVAL PATHWAY

HYPOTHESIS:

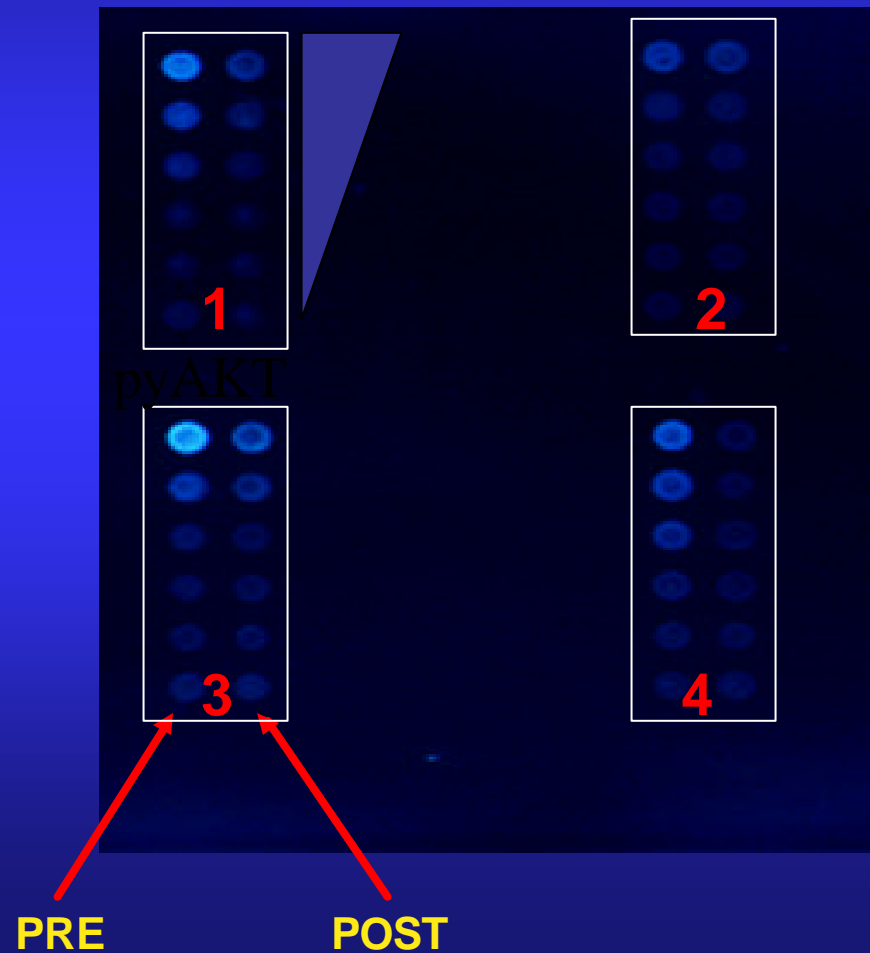
- Increased Sensitivity to apoptosis inducing therapy (e.g. Taxol)
- Suppression of growth through de-repression of p21(Cip1/WAF)

Proteomic Endpoints from Clinical Trial Biopsies: Use of Protein Arrays

Pre and Post HERCEPTIN (1 Month)

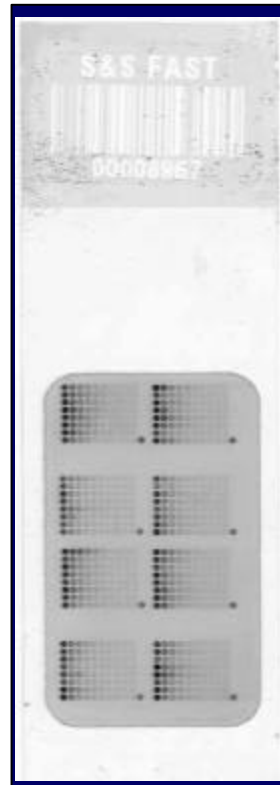
- Phospho-AKT Endpoint
- 500 microdissected cells
- Pre and Post Treatment Studies

Responders: 1,3,4
Non-Responder: 2

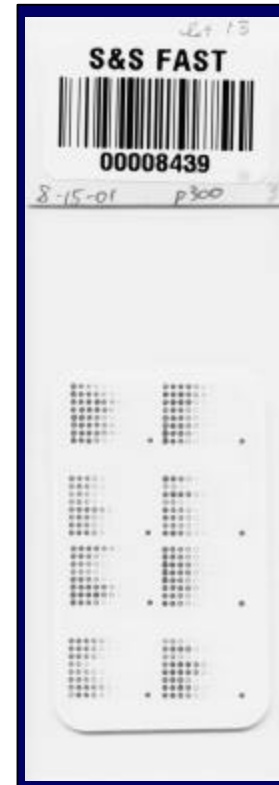


Protein array specification

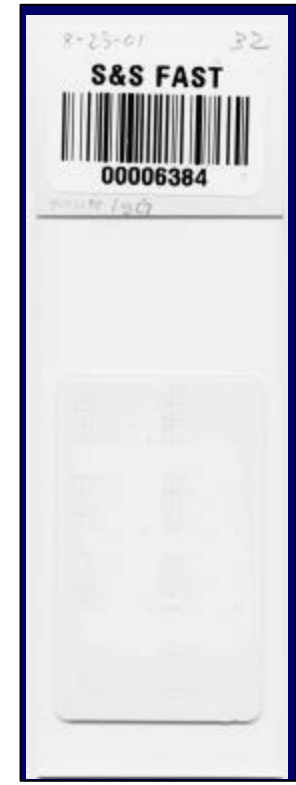
- Schleicher & Schuell (www.s-and-s.com) FAST slide (glass slide embedded 21 x 35 mm nitrocellulose membrane) was used.
- Total number of spots is 648.
- Spot all NCI60 cell lysates and 4 pools on a single slide.
- Each cell line has 10 different concentration spots.
- Achieved more than 1000-fold dynamic range.
- Requires total protein and negative control stains for a protein expression measurement.
- Takes 5 hours for making 20 full arrays.



Total protein (stained by SYPRO RUBY)



Protein of interest (p300)



Negative control (mouse IgG)

Raw pixel data generation by P-SCAN

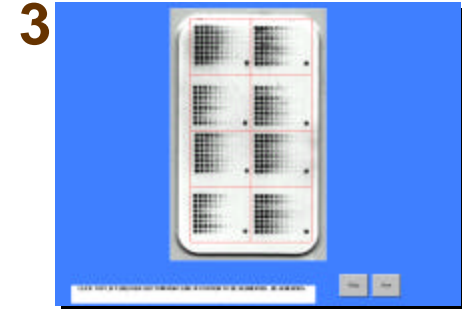
P-SCAN (Peak quantification using Statistical Comparative Analysis) is available at <http://abs.cit.nih.gov/pscan/>



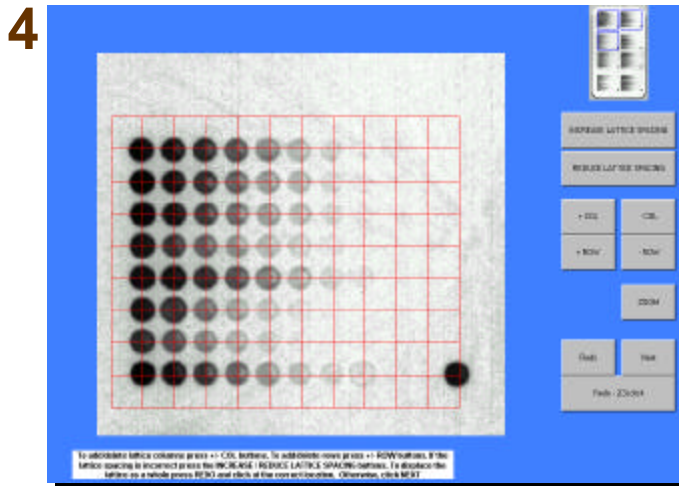
Apply an image (TIFF) and select the area of array.



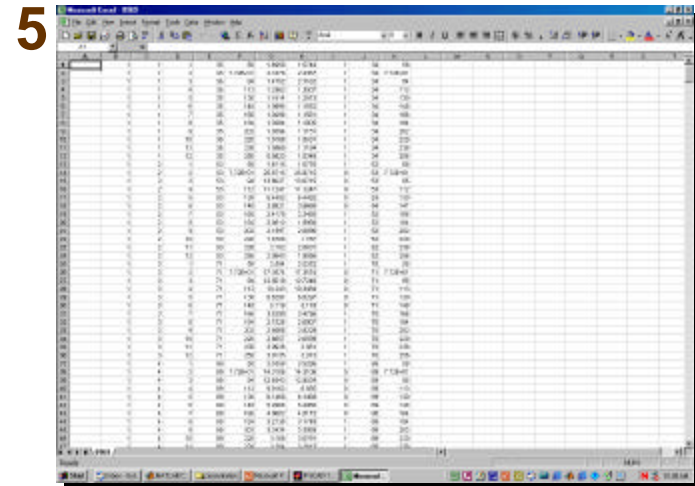
The array has been selected.



Set up the field. Intensity reading will be carried out by each field. There are $2 \times 4 = 8$ fields above.



Align the lattice. A total of 120 intersections will set in a field and generates intensity number per spot. The right bottom dark spot is for control the alignment.



Raw pixel intensity data is exported onto an Excel worksheet along with its address on the array.